

Claims

1. A method for selecting siRNA comprising selecting an siRNA molecule of 19 – 25 nucleoside bases, said method comprising:
 - (a) selecting a target gene;
 - 5 (b) measuring the functionality of sequences of 19 – 25 nucleotides in length that are substantially complementary to a stretch of nucleotides of the target sequence, wherein said functionality is dependent upon non-target specific criteria.
- 10 2. The method according to claim 1 wherein said functionality is determined by applying one of the following formulas:

—

Formula I = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

15 Formula II = $-(GC/3) - (AU_{15-19})*3 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula III = $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C})*3$;

20 Formula IV = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C})*2 - (G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

Formula V = $-(G_{13})*3 - (G_{19}) + (A_{19})*2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$;

25 Formula VI = $-(G_{13})*3 - (C_{19}) + (A_{19})*2 + (A_3)$;

Formula VII = $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C})*1 - (G_{13})*3 - (C_{19}) + (A_{19})*3 + (A_3)*3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$;

30

wherein in Formulas I – VII:

$AU_{15-19} = 0 - 5$ depending on the number of A or U bases on the sense

strand at positions 15–19;

$G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise its value is 0;

5

$GC =$ the number of G and C bases in the entire sense strand;

$Tm_{20^\circ C} = 1$ if the Tm is greater than $20^\circ C$;

$A_3 = 1$ if A is the base at position 3 on the sense strand, otherwise its value is 0;

10

$A_{11} = 1$ if A is the base at position 11 on the sense strand, otherwise its value is 0;

$A_{14} = 1$ if A is the base at position 14 on the sense strand, otherwise its value is 0;

15

$A_{19} = 1$ if A is the base at position 19 on the sense strand, otherwise its value is 0;

$U_5 = 1$ if U is the base at position 5 on the sense strand, otherwise its value is 0;

$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;

20

or,

Formula VIII: $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-$
25 $9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}$

$+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+$
 $11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-$
30 $30*X;$ and

Formula IX: $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*$

30 $C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+$
 $(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-$
 $10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-$
 $30*X$

wherein

$A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;
 $A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;
 $A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;
 $A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;
5 $A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;
 $A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;
 $A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;
 $A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;
 $A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;
10 $A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;
 $A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;
15 $C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;
 $C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;
 $C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;
 $C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;
 $C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;
20 $C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;
 $C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;
 $C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

25 $G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;
 $G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;
 $G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;
 $G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;
 $G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;
30 $G_{19} = 1$ if G is the base at position 19 of the sense strand, otherwise if another base
is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$ if U is the base at position 1 on the sense strand, otherwise its value is 0;
 $U_2 = 1$ if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;
 $U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;
 $U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;
 $U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;
5 $U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0;
 $U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;
 $U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;
 $U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;
 $U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

10

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand
or within positions 15 – 18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

$Tm = 100$ if the targeting site contains an inverted repeat longer than 4 base pairs,
15 otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a
row.

20 3. A method of gene-silencing comprising selecting an siRNA according to
claim 2 and introducing it into a cell.

4. The method according to claim 3 wherein said introducing is by allowing
passive uptake of the siRNA. ~~.....~~

25 5. The method according to claim 3, wherein said introducing is through the
use of a vector.

6. A method for developing an siRNA algorithm for selecting siRNA, said
method comprising:

30 (a) selecting a set of siRNA;
 (b) measuring the gene silencing ability of each siRNA from said set;
 (c) determining the relative functionality of each siRNA;
 (d) determining the amount of improved functionality by the presence or
 absence of at least one variable selected from the group consisting of

the total GC content, melting temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and

5 (e) developing an algorithm using the information of step (d).

7. A method of selecting an siRNA with improved functionality, said method comprising using the algorithm of claim 6.

10 8. A method of selecting hyperfunctional siRNA, said method comprising using at least one functional siRNA, wherein at least one said functional siRNA has been selected according to the method of claim 7 and measuring the silencing ability of said at least one functional siRNA, wherein silencing ability is measured at a concentration of less than 1
15 nanomolar siRNA.

9. An siRNA molecule, wherein said siRNA molecule is effective at silencing Bcl-2.

20 10. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sequence substantially similar to a sequence selected from the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCAUUUAAG (SEQ. ID NO. 302);
GUACGACAACCGGGAGAUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
25 UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
CAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);
30 GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

11. The siRNA molecule of claim 10, wherein said siRNA molecule comprises a sequence selected from the group consisting of

GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCAGGGAGAUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
5 UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);
GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
10 GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

12. The siRNA molecule of claim 11, wherein said siRNA molecule comprises GCAUGCGGCCUCUGUUUGA .
- 15 13. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a sense strand and an anti-sense strand.
14. The siRNA molecule of claim 9, wherein said siRNA molecule comprises a hairpin.
- 20 15. The siRNA molecule of claim 9, wherein said siRNA molecule comprises between 18 and 30 base pairs.
- 25 16. A kit for gene silencing comprising at least one siRNA selected from the group consisting of sequences substantially similar to the group consisting of GGGAGAUAGUGAUGAAGUA (SEQ. ID NO. 301);
GAAGUACAUCCAUUAUAAG (SEQ. ID NO. 302);
GUACGACAACCAGGGAGAUA (SEQ. ID NO. 303);
AGAUAGUGAUGAAGUACAU (SEQ. ID NO. 304);
UGAAGACUCUGCUCAGUUU (SEQ. ID NO. 305);
GCAUGCGGCCUCUGUUUGA (SEQ. ID NO. 306);
30 UGCGGCCUCUGUUUGAUUU (SEQ. ID NO. 307);
GAGAUAGUGAUGAAGUACA (SEQ. ID NO. 308);

GGAGAUAGUGAUGAAGUAC (SEQ. ID NO. 309); and
GAAGACUCUGCUCAGUUUG (SEQ. ID NO. 310).

17. A method of gene silencing comprising using the siRNA molecule of
5 claim 10.

18. A method of gene silencing comprising using the siRNA molecule of claim
11.

10 19. A kit, wherein said kit is comprised of at least two siRNA, wherein said at
least two siRNA comprise a first optimized siRNA and a second optimized
siRNA, wherein said first optimized siRNA and said second optimized
siRNA are optimized according to one of the following formulas:

15 Formula I = -(GC/3) +(AU₁₅₋₁₉) -(Tm_{20°C})*3 -(G₁₃)*3 -(C₁₉) +(A₁₉)*2 +(A₃)
+(U₁₀)+(A₁₄) -(U₅) -(A₁₁);

Formula II = -(GC/3) -(AU₁₅₋₁₉)*3 -(G₁₃)*3 -(C₁₉) +(A₁₉)*2 +(A₃);

20 Formula III = -(GC/3) +(AU₁₅₋₁₉) -(Tm_{20°C})*3;

Formula IV = -(GC/2)+(AU₁₅₋₁₉)/2-(Tm_{20°C})*2 -(G₁₃)*3 -(C₁₉) +(A₁₉)*2
+(A₃) +(U₁₀)+(A₁₄) -(U₅) -(A₁₁); ~~.....~~

25 Formula V = -(G₁₃)*3 -(C₁₉) +(A₁₉)*2 +(A₃) +(U₁₀)+(A₁₄) -(U₅) -(A₁₁);

Formula VI = -(G₁₃)*3 -(C₁₉) +(A₁₉)*2 +(A₃);

30 Formula VII = -(GC/2) +(AU₁₅₋₁₉)/2 -(Tm_{20°C})*1 -(G₁₃)*3 -(C₁₉) +(A₁₉)*3
+(A₃)*3 +(U₁₀)/2+(A₁₄)/2 -(U₅)/2 -(A₁₁)/2;

wherein in Formulas I – VII:

AU₁₅₋₁₉ = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 –19;

5 G₁₃ = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

C₁₉ = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

T_{m 20°C} = 1 if the T_m is greater than 20°C;

10 A₃ = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

A₁₁ = 1 if A is the base at position 11 on the sense strand, otherwise its value is 0;

A₁₄ = 1 if A is the base at position 14 on the sense strand, otherwise its value is 0;

15 A₁₉ = 1 if A is the base at position 19 on the sense strand, otherwise its value is 0;

U₅ = 1 if U is the base at position 5 on the sense strand, otherwise its value is 0;

20 U₁₀ = 1 if U is the base at position 10 on the sense strand, otherwise its value is 0;

or,

25 Formula VIII: (-14)*G₁₃-13*A₁-12*U₇-11*U₂-10*A₁₁-10*U₄-10*C₃-10*C₅-10*C₆-9*A₁₀-9*U₉-9*C₁₈-8*G₁₀-7*U₁-7*U₁₆-7*C₁₇-7*C₁₉+7*U₁₇+8*A₂+8*A₄+8*A₅+8*C₄+9*G₈+10*A₇+10*U₁₈+11*A₁₉+11*C₉+15*G₁+18*A₃+19*U₁₀-T_m-3*(GC_{total}) - 6*(GC₁₅₋₁₉)-30*X; and

30

Formula IX: (14.1)*A₃+(14.9)*A₆+(17.6)*A₁₃+(24.7)*A₁₉+(14.2)*U₁₀+(10.5)*C₉+(23.9)*G₁+(16.3)*G₂+(-12.3)*A₁₁+(-19.3)*U₁+(-12.1)*U₂+

$$(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{\text{total}})-6*(GC_{15-19})-30*X$$

wherein

5 $A_1 = 1$ if A is the base at position 1 of the sense strand, otherwise its value is 0;
 $A_2 = 1$ if A is the base at position 2 of the sense strand, otherwise its value is 0;
 $A_3 = 1$ if A is the base at position 3 of the sense strand, otherwise its value is 0;
 $A_4 = 1$ if A is the base at position 4 of the sense strand, otherwise its value is 0;
 $A_5 = 1$ if A is the base at position 5 of the sense strand, otherwise its value is 0;
10 $A_6 = 1$ if A is the base at position 6 of the sense strand, otherwise its value is 0;
 $A_7 = 1$ if A is the base at position 7 of the sense strand, otherwise its value is 0;
 $A_{10} = 1$ if A is the base at position 10 of the sense strand, otherwise its value is 0;
 $A_{11} = 1$ if A is the base at position 11 of the sense strand, otherwise its value is 0;
 $A_{13} = 1$ if A is the base at position 13 of the sense strand, otherwise its value is 0;
15 $A_{19} = 1$ if A is the base at position 19 of the sense strand, otherwise if another base
 is present or the sense strand is only 18 base pairs in length, its value is 0;

20 $C_3 = 1$ if C is the base at position 3 of the sense strand, otherwise its value is 0;
 $C_4 = 1$ if C is the base at position 4 of the sense strand, otherwise its value is 0;
 $C_5 = 1$ if C is the base at position 5 of the sense strand, otherwise its value is 0;
 $C_6 = 1$ if C is the base at position 6 of the sense strand, otherwise its value is 0;
 $C_7 = 1$ if C is the base at position 7 of the sense strand, otherwise its value is 0;
 $C_9 = 1$ if C is the base at position 9 of the sense strand, otherwise its value is 0;
 $C_{17} = 1$ if C is the base at position 17 of the sense strand, otherwise its value is 0;
25 $C_{18} = 1$ if C is the base at position 18 of the sense strand, otherwise its value is 0;
 $C_{19} = 1$ if C is the base at position 19 of the sense strand, otherwise if another base
 is present or the sense strand is only 18 base pairs in length, its value is 0;

30 $G_1 = 1$ if G is the base at position 1 on the sense strand, otherwise its value is 0;
 $G_2 = 1$ if G is the base at position 2 of the sense strand, otherwise its value is 0;
 $G_8 = 1$ if G is the base at position 8 on the sense strand, otherwise its value is 0;
 $G_{10} = 1$ if G is the base at position 10 on the sense strand, otherwise its value is 0;
 $G_{13} = 1$ if G is the base at position 13 on the sense strand, otherwise its value is 0;

$G_{19} = 1$ if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$ if U is the base at position 1 on the sense strand, otherwise its value is 0;

5 $U_2 = 1$ if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$ if U is the base at position 3 on the sense strand, otherwise its value is 0;

$U_4 = 1$ if U is the base at position 4 on the sense strand, otherwise its value is 0;

$U_7 = 1$ if U is the base at position 7 on the sense strand, otherwise its value is 0;

$U_9 = 1$ if U is the base at position 9 on the sense strand, otherwise its value is 0;

$U_{10} = 1$ if U is the base at position 10 on the sense strand, otherwise its value is 0

$U_{15} = 1$ if U is the base at position 15 on the sense strand, otherwise its value is 0;

$U_{16} = 1$ if U is the base at position 16 on the sense strand, otherwise its value is 0;

$U_{17} = 1$ if U is the base at position 17 on the sense strand, otherwise its value is 0;

$U_{18} = 1$ if U is the base at position 18 on the sense strand, otherwise its value is 0;

15

GC_{15-19} = the number of G and C bases within positions 15 – 19 of the sense strand
 or within positions 15 – 18 if the sense strand is only 18 base pairs in length;

GC_{total} = the number of G and C bases in the sense strand;

Tm = 100 if the targeting site contains an inverted repeat longer than 4 base pairs,

20

X = the number of times that the same nucleotide repeats four or more times in a row.